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Vaginal Bacterial Flora of Nigeria Local Bitches During Different Stages of Reproductive Cycle

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ABSTRACT

From a total of 41 local bitches in different stages of the reproductive cycle (immature=10, Anoestrus=6, Proestrus=5, Oestrus=7, Pregnant=7, and Dioestrus-Non pregnant=6), 44 bacterial organisms were isolated. These isolates include; *Staphylococcus* spp., *Corynebactirum* spp., *Klebsiella* spp. and *Streptococcus* spp. *Staphylococcus* spp. (40.91%) and *Corynebacterium* spp. (29.54%) have been isolated in all the stages of the reproductive cycle. These organisms can, therefore, be considered as a part of the normal vaginal flora of the Nigerian local bitch.

Key Words: Bacterial flora; Bitches; Reproductive cycle

INTRODUCTION

Many bacterial organisms have been isolated from the canine vagina. The type of organisms isolated may vary with the age of bitch (Olson & Martha, 1978). A higher percentage of prepuberal bitches have been suggested to have coagulase-positive *Staphylococcus* than do post puberal bitches (Feldman, 1989). The commonly isolated organisms include *E. coli*, *Staphylococcus* spp., *Streptococcus* spp., *Pasteurella* spp., *Proteus* spp., *Bacillus* spp., *Corynebacterium* spp., *Pseudomonas* spp. and *Micrococcus* spp. Others include *Neisseria* spp., *Klebsiella* spp. and *Moraxella* spp. (Hirsh & Wiger, 1977; Olson & Martha, 1978; Allen & Dagnall, 1982; Olson *et al.,* 1986; Gandotra *et al.,* 1994). Some of these organisms have been cultured from healthy as well as infertile bitches (Osbaldiston *et al.,* 1972). Most normal bitches have bacterial flora present in the anterior vagina and similar types of aerobic bacteria are present in the vaginal vault of infertile bitches (Feldman, 1989).

There are no qualitative differences in the types of bacteria isolated from normal prepuberal, oophorectomised or intact mature bitches, nor between the anterior and posterior vagina, nor among the various stages of the oestrus cycle (Olson & Martha, 1978; Johnson, 1991). Frequently, vaginal cultures are obtained in an attempt to correlate various diseases (e.g. inflammation of the clitoral fossa, vestibule, vagina, cervix or uterus; infertility, abortion, neonatal deaths) with specific infectious agents (Olson *et al.,* 1986), but it is difficult to associate disease with a specific bacterial isolate since a variety of organisms are present in the vagina of bitches with or without reproductive diseases (Olson & Martha, 1978).

It has also been reported that the type of bacteria isolated do not appear to vary with the different stages of the oestrous cycle, but an increased number of organisms appear to be present during proestrus and oestrus (Allen & Dagnall, 1982; Baba *et al.,* 1983). In a recent study, Gandotra *et al.* (1994), isolated *Staphylococcus aureus* in 38.47% and *Streptococcus* spp. in 30.77%, *Corynebacterium pyogenes* in 23.07% and *E. coli* in 7.59% in 24 cases of canine pyometra. In another study, vaginal cultures from young and mature bitches with chronic vaginitis yielded mixed populations of *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Proteus* spp., *Pasteurella* spp., *Citrobacter* spp. and *Enterobacter* spp. These organisms are qualitatively the same as the normal bacterial flora of the canine vagina (Johnson, 1991).

Bacterial infections have been implicated as a cause of infertility in the bitch. These infections are thought to be sub-clinical in the infertile bitch, but occasionally result in obvious vaginitis, metritis, pyometra, or systemic infections. This work, therefore, seeks to generate data on the vaginal bacterial flora of Nigerian local bitches during the different stages of the reproductive cycle.

MATERIALS AND METHODS

Vaginal swab samples were obtained from 41 local bitches within the Maiduguri Metropolis. Bitches sampled were in different stages of the reproductive cycle and of varying ages. Among the bitches sampled, 10 were prepuberal (6-7 months), six were in anoestrous, five in proestrus, seven in oestrus, seven pregnant, while six were in dioestrus (non-pregnant). Samples were taken during the peak periods in proestrous, oestrous, dioestrus and about day 45 in pregnant bitches. Stages of the reproductive cycle were determined based on history, physical examination, careful observation of behavioral changes in the bitches. Animals were adequately restrained on lateral recumbency on an examination table. The perivaginal area was scrubbed with a potent antiseptic (TCP, Pfizer products). Sterile swab sticks (Evepon Sterile Swabs, Nigeria) were passed into the vagina with the vaginal labia parted. The stick was directed cranio-dorsally initially (avoiding the clitoral fossa and the uretheral orifices and then longitudinally through a length of about 12 cm as described by Olson *et al.* (1984) and Holst (1986). The swab was then rolled around the vaginal wall

gently and withdrawn carefully into its adequately labeled sterile container for transport to the laboratory. The swab was then inoculated onto 5% sheep blood and McConkey agar and the plates incubated at 37ºC for 24-48 hours after which discrete colony growths were observed and recorded. Gram staining and other routine biochemical tests were further employed in the identification of bacteria based on standard procedures (Carter, 1975; Cheesbrough, 1994).

RESULTS AND DISCUSSION

A total of 44 bacterial organisms were isolated from the vagina of 41 bitches. These isolates were distributed among five genera of bacteria (Table I). The common isolates encountered were *Staphylococcus* spp. and *Corynebacterium* spp., which accounted for 70.45% of the total isolates. *Proteus* spp. accounted for 13.64%; while *Klebsiella* spp. and *Streptococcus* spp. accounted for 15.9%. *Staphylococcus* spp. and *Corynebacterium* spp. were commonly isolated throughout the oestrous cycle. *Proteus* spp. were isolated mainly among immature, oestrous and non-pregnant dioestrous bitches. *Klebsiella* spp. were isolated only from oestrous and pregnant bitches, with 75% of the isolates from pregnant bitches. *Streptococcus* spp. were isolated from immature, anoestrous and non-pregnant dioestrous bitches.

The highest rate of isolation of bacteria per dog was recorded among the non-pregnant dioestrous bitches (1.5), followed by pregnant bitches (1.3), anoestrous bitches (1.2), immature bitches (0.9) and proestrous bitches (0.4). The bacteria isolated from immature bitches include *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp. and *Proteus* spp., totaling 25% of the bacterial isolates. Pregnant bitches yielded *Staphylococcus* spp., *Klebsiella* spp., *Corynebacterium* spp., constituting 20.45% of the total bacterial isolates. Among the anoestrous bitches, *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. accounting for 15.9% were the bacteria isolated. Another 13.64% of the bacteria isolated were from oestrous bitches with *Staphylococcus* spp., *Corynebacterium* spp., *Klebsiella* spp. and *Proteus* spp., being the common isolates. Dioestrous bitches were found to harbour *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Proteus* spp., constituting 20.45% of the total bacterial isolates. During proestrus, *Staphylococcus* spp. and *Corynebacterium* spp. (4.5%) were isolated. A total of three bitches each in proestrus and oestrus did not give any growth.

The commonest isolates were *Staphylococcus* spp. (40.9%) and *Corynebacterium* spp. (29.54%). These organisms including *Proteus* spp. (13.64%), *Klebsiella* spp. (9.09%), and *Steptococcus* spp. (6.82%) were isolated from bitches during different stages of the reproductive cycle. These organisms could, therefore, be considered as a part of the normal vaginal bacterial flora of the Nigerian local bitch.

Non-pregnant dioestrous bitches appear to harbour more bacteria with an isolation rate of 1.5, followed by pregnant bitches with 1.3. The high isolation rate recorded among dioestrous and pregnant bitches can be related to the prevailing endocrine status of the animal. This is because progesterone is said to promote conditions suitable for bacterial growth within the genital tract and low pH (Amin, *et al.,* 1996). There also may likely exist an immuno-suppressive effect during the luteal phase, which allows for the proliferation of these bacterial organisms.

The lowest rate of isolation was recorded in the proestrous bitches. This can be associated with the proestrous irrigation of the vaginal lumen by uterine fluid. The reports of Allen and Dagnall (1982) and Baba *et al.* (1983) that an increased number of organisms appear to be present during proestrus and oestrus could not be sustained by the findings of this study, because more bacterial isolates were found to be present during dioestrus and anoestrus than during proestrus and oestrus. This finding agrees with the report of Holst (1986).

Gandotra *et al.* (1994) reported the isolation of 38.47% *Staphylococcus aureus*, 30.77% *Staphylococcus* spp., 20.07% *Corynebacterium* spp. and 7.69% *E. coli* from cases of pyometra; and Johnson (1991) in a study of chronic vaginitis, isolated bacterial agents including pure cultures of *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *Proteus* spp. among others. However, in this study, *Klebsiella* spp., *Staphylococcus* spp., *Corynebacterium* spp and *Proteus* spp. were also isolated from clinically healthy

Table I. Distribution of Vaginal bacterial isolates over oestrous cycle and pregnancy in 41 dogs

Cyclic stage	No. of dogs	Type of isolates					No. of isolates	Isolates per dog
		Staphylococcus spp.	*Corynebacterium* spp.	*Klebsiella* spp.	*Streptococcus* spp.	*Proteus* spp.		
Immature	10	6	2	–	1	2	11	1.1
Anoestrus	6	2	4	–	1	–	7	1.2
Proestrus	5	1	1	–	–	–	2	0.4
Oestrus	7	1	3	1	–	1	6	0.9
Pregnant	7	5	1	3	–	–	9	1.3
Dioestrus (non pregnant)	6	3	2	–	1	3	9	1.5
Total	41	18	13	4	3	6	44 (1.1 isolate per dog)	

Note: *Staphylococcus* spp isolated were all coagulase-negative

bitches during oestrus. This, therefore, shows that the mere isolation of bacterial agents in the presence of an on-going genital disease may not necessarily be incriminative of that organisms. This finding is in agreement with the report of Olson *et al.* (1986).

However, bacterial isolation in diseased or healthy bitches is important because opportunistic bacteria can become pathogenic when an enabling condition is created. Most of the isolates in this study are opportunistic. In the presence of any form of trauma, they may invade the genitalia giving rise to genital inflammatory conditions and septicemia. Their isolation, therefore, can be of great significance in the clinical diagnosis and management of reproductive diseases in the bitch.

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